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(54) Title: PAPILLOMAVIRUS E7 PROTEIN

(57) Abstract

Papillomavirus E7 protein from HPV or BPV is effective therapeutically in the regression (but not prevention) of papillomavirus tumours in mammals. Preferably the protein or fragment thereof is in the form of a coprotein with beta-galactosidase or GST. Preferred antigenic protein fragment sequences are also described.

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PAPILLOMAVIRUS E7 PROTEIN

TECHNICAL FIELD

The present invention relates to the use of papillomavirus E7 protein in medicine, particularly for the regression of papillomavirus disease tumours particularly in mammals including humans; and to pharmaceutical formulations comprising the E7 protein.

BACKGROUND OF THE INVENTION

Papillomaviruses induce a variety of lesions both in humans and in animals. Some papillomas, albeit benign, are themselves a clinical problem, such as laryngeal papillomas of children (Steinberg and Abramson, 1985) or penile papillomas of bulls (Jarrett, 1985a), and others are known to be a risk factor in the pathogenesis of cancer, as in the case of flat lesions of the cervix or penile condylomata in humans (zur Hausen, 1978) and papillomas of the alimentary canal in cattle (Jarrett et al, 1978). In particular, human papillomavirus types HPV-16 and HPV-18 and bovine papillomavirus type 4 are potentially carcinogenic. Therefore both in human and veterinary medicine an antiviral vaccine, particularly a therapeutic one inducing lesion rejection, would be of major importance. Vaccination studies in humans present several problems: first of all experimentation is ethically unacceptable and, secondly, very limited amounts

of virus are available as some lesions, in particular those of the cervix, do not produce viral progeny, and no in vitro system is yet available which allows vegetative replication of virus. The production of viral proteins in bacteria and the use of synthetic peptides have circumvented this last problem and have allowed the ongoing analysis of the immune response to papillomavirus infection (see for instance Jenison et al, 1988: Jochmus-Kudielka et al, 1989; Tindle et al, 1990, Dillner, 1990 and Strang et al, 1990). Whilst investigation into the feasibility of a human papillomavirus vaccine is still at an early stage, effective prophylactic vaccines, both natural (Jarrett et al, 1990a) and genetically engineered (Pilachinski et al, 1986; Jarrett et al, 1991) have already been produced against bovine papillomaviruses, and regression of Shope papillomas has been achieved by vaccinating rabbits with tumour tissue extracts (Evans et al, 1962). The bovine system is an excellent model for the human one, given the several similarities between the two: multiple virus types with high lesion specificity (Campo et al, 1981; Jarrett et al, 1984), homology of genetic structure (Danos et al, 1984) and progression of some lesions to malignancy (Jarrett et al, 1978). The bovine system also presents several advantages: cofactors in oncogenesis are known (Jarrett et al, 1978; Campo and Jarrett, 1986) and, above all, direct experimentation is possible (Jarrett, 1985a).

It has recently been shown that vaccination with

bovine papillomavirus type 2 (BPV-2) successfully prevented infection by the same virus (Jarrett et al, 1990a), but not by other virus types (Jarrett et al,1990b). Prevention was accompanied by production of neutralising antibodies in the serum of vaccinated animals, indicating that neutralising epitopes are present in the virus.

Our earlier British patent application GB9113809.9 discloses therapeutic activity of L2 protein of bovine papilloma virus BPV-2 (Jarrett et al, 1991; Campo, 1991).

International patent publication W092/05248 discloses the use of the E7 protein of human papilloma virus HPV.

SUMMARY OF THE INVENTION

Generally speaking, the present invention resides in the discovery that the papillomavirus E7 protein is therapeutically effective in the treatment of papillomavirus disease and particularly for the regression of tumours.

Thus, the present invention provides the use of papillomavirus E7 protein in medicine, particularly for the therapy of papillomavirus disease.

The invention also provides a pharmaceutical formulation for the therapy of papillomavirus disease, which comprises; papillomavirus E7 protein in admixture with a pharmaceutically acceptable carrier.

The invention further provides papillomavirus E7 protein for use in the production of a medicament for use in medicine, particularly for use in the therapy of

papillomavirus disease.

The invention still further provides a method of treating a mammal for the therapy of papillomavirus disease, which comprises the administration of papillomavirus E7 protein to the mammal.

The E7 protein has been found to be antigenic and to lead to the production of antibodies. However, no protective effect against viral infection and tumour production is observed. Instead, the protein is found to have a therapeutic effect in reducing the size of tumours and speeding their regression.

Generally speaking, the therapeutic effect of the E7 protein is limited to the respective papillomavirus type. Thus, for general therapeutic applications, especially where the particular papillomavirus type is unknown, it may be desirable to employ a mixture of E7 proteins from a variety of papillomavirus types.

Generally, the therapy will be applicable to papillomavirus infections of mammals, including humans and animals. The present work has been done on E7 protein of bovine papillomavirus BPV-4 (which generally affects the alimentary canal; Campo et al, 1980). However, because of substantial sequence homology between BPV-4 and human papillomavirus HPV-16 (see Figures 1 and 2; Jaggar et al, 1990) it can be predicted that E7 protein will also demonstrate therapeutic effectiveness in tumour regression against HPV-16 and possibly HPV-18 human carcinogenic viral types. In this way pre-cancerous lesions may be

treated. In animals, the therapy is useful for the regression of tumours, for example the removal of warts from the udders (BPV-2) or mouth (BPV-4) of milk cows, and for the treatment of horses and donkeys.

The E7 protein is generally produced by recombinant DNA techniques. In particular, a plasmid containing the gene coding for the E7 protein may be transfected into a bacterium such as E. coli and cultured. The entire E7 protein as it exists in nature may be employed, or an antigenic fragment or fragments thereof may be used providing that the antigenicity and therapeutic effectiveness is retained.

In particular the antigenic fragment may be an immunogenic epitope. Immunodominant B and T cell epitopes are shown in Figure 4. B1, B2 and B3 map to amino acids 1-30, 79-98 and 51-69 respectively. T1 and T2 map to amino acids 20-50 and 70-88 respectively. The whole of the E7 protein or epitope may be employed, or with additions deletions or substitutions which do not substantially effect its therapeutic effectiveness.

The E7 protein is preferably administered as a fusion protein, for example fused to beta-galactosidase or glutathione S-transferase (GST). This stabilises the E7 protein and may assist purification thereof. The co-protein may also be antigenic and assist in providing general stimulation of the immune system.

The E7 protein will usually be administered in the

form of a pharmaceutical formulation. The formulation contains a pharmaceutically acceptable carrier. The carrier must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Since the protein is broken down in the stomach, oral administration is not preferred. The pharmaceutical formulation is preferably formulated for parenteral administration, including subcutaneous, intramuscular and intravenous injection; or as a suppository or pessary. For parenteral administration the formulation may be presented as a sterile solution or suspension in a suitable liquid vehicle, which may also contain preservatives and materials for rendering the formulation isotonic. The formulations may be presented in unit-dose or multi-dose containers. The carrier will generally be apyrogenic. Each dose will generally contain 100 to 10,000 micrograms of the E7 protein.

In order to enhance the therapeutic effect of the protein, it may be administered together with an adjuvant, such as Freund's incomplete adjuvant, as an oil-in-water emulsion or using other adjuvant systems known in the art such as L101 and DDA as used in Pilacinksi et al (1986). Aluminium gel (made up for example from equal volumes of 3% aluminium hydroxide and 2% aluminium phosphate) may also be an advantageous adjuvant system.

DESCRIPTION OF PREFERRED EMBODIMENTS.

Embodiments of the present invention will now be described by way of example only with reference to the following experimental protocol.

The attached drawings show the following.

Figure 1 shows the genomic organisation of BPV-4 and for comparison HPV-16.

The percentages amino acid homology are:

E1 = 55.3

E2 = 43.6

E4 = 32.6

E7 = 53.6

L1 = 66.0

L2 = 47.8

The BPV-4 genome is 7265 base pairs long; the HPV-16 genome is 7904 base pairs long. The open reading frames are represented by boxes. E1-8=early genes; L1-2=late genes.

Figure 2 shows the homology between the E7 proteins of BPV-4 and HPV-16. Identical amino acids are linked by a continuous line and similar amino acids by a broken line. Region 1 and 2=retinoblastoma protein binding domain; Zn^{++}= zinc fingers domains; hydrophobic = stretch of hydrophobic amino acids;

Figure 3 shows the results of vaccination with BPV-4 E7 protein in terms of the development of papillomas of size greater than 2 mm in control animals and in

vaccinated animals. The mean tumour number of papillomas per animal is plotted against weeks after challenge; and

Figure 4 shows the B and T cell epitopes of BPV-4 and E7 protein as mapped.

EXAMPLE 1 (Production of BPV-4 E7 and vaccination)

MATERIALS AND METHODS

Calves

Nineteen 20-week old calves of several different breeds were obtained from a papilloma-free source. They were randomly assigned to two groups, group A of eight animals and group B of eleven animals. They were housed in separate, clean, well ventilated pens in the isolation unit of the Department of Veterinary Pathology, Glasgow. All the calves were bled on arrival for haematological analysis and to obtain pre-inoculation serum samples. The experiment was started when the calves were approximately 26 weeks old.

Production of E7 peptide in Escherichia coli

The open reading frame (ORF) encoding the E7 peptide was isolated by digesting the BPV-4 genome cloned in pAT 153 (Campo and Coggins, 1982) with Bam HI. The open reading frame encoding the E7 peptide (nt 647-1009 of the BPV-4 genome; Jackson et al, 1991) was isolated as a fragment encompassing nt 652-1250. The fragment contains the E7

ORF ATG and termination codons. The fragment was cloned in the pUR and pGEX vector series (Ruther and Muller-Hill, 1983); Smith and Johnson, 1988) giving rise to plasmids pURE7 and pGEXE7, which produce beta-galactosidase (b-gal)-E7 fusion and glutathione S-transferase (GST)-E7 fusion proteins respectively. The plasmids produced were transfected into E. Coli JM 109. Peptide for vaccination was prepared from mid-log phase cultures induced for 1 hours in L-broth supplemented with 100 ug/ml ampicillin and containing 1mM IPTG. Bacterial pellets resuspended in lysozyme buffer (50mM TRIS-HC1 pH 8.0, 10mM $McC1_2$, 50mM glucose, 1 mg/ml lysozyme) were left at 20°C for 10 min, when EDTA was added to 50mM. Following cell lysis by the addition of Triton X100 to 1% (v/v), the respective fusion peptide was pelleted at 39000 g for 30 min and resuspended by boiling and sonication in 5% SDS, 50mM B-mercaptoethanol, 50mM TRIS-HC1, pH 8.0 Purity of 90-95% was achieved by preparative SDS PAGE, the final yields being up to 2 mg of product per gm wet weight of cells. The protein was stored at -20°C before use, but prolonged storage caused degradation. In this way, (beta-gal) -E7 and (GST) -E7 fusion proteins were produced.

Experimental design

The eleven animals in group A were vaccinated with E7, while the eight animals in group B were kept as controls.

Vaccination (group A)

The calves receiving the E7 vaccine were given a 1ml PBS suspension containing 1 mg of either of the two E7 fusion proteins plus 1 ml of Freund's incomplete adjuvant into the right quadriceps muscle. This was repeated four weeks later as a boost.

Virus challenge

BPV-4 was purified from alimentary papillomas (Campo et al, 1980) and the concentration of viral particles was estimated by the electron microscope assay (Jarrett et al, 1990a). Each calf was challenged at ten sites in the palate in two parallel rows of five sites each with 10^{11} virus particles four weeks after vaccination.

RESULTS

Characterization of fusion protein

The b-gal -E7 and the GST-E7 fusion proteins were characterized immunologically. They were injected into rabbits or mice and the antisera were tested against the fusion proteins in Western blot assays. The antisera were reactive with both types of fusion proteins. Therefore the fusion proteins present their immunodominant epitopes effectively to the host immune system.

Vaccination with E7 inhibits papilloma growth and promotes tumour rejection.

All the eleven vaccinated animals were vaccinated before challenge with virus. The vaccinated animals had high titre anti-E7 antibodies in their sera before virus injection, whereas the control animals developed anti-E7 antibodies only thirteen weeks post challenge and the magnitude of their response was much smaller than in the vaccinated animals. Likewise, E7-activated T-lymphocytes were detected in the vaccinated animals much earlier than in the controls, and in addition the response of control T-lymphocytes to E7 stimulation was very sluggish. These results confirm the immunological activity of E7 fusion proteins also in cattle.

Following challenge, all animals developed tumours at the ten sites of injection, showing that the virus was equally efficatious in both sets of animals and that all animals were equally susceptible to virus infection. In other words, no protection effect was seen with the E7 vaccine. However, fifteen weeks after challenge, most papillomas developed to full size and actively produced virus in the control animals. In contrast, in the vaccinated animals on average only one papilloma per animal achieved full size and the papillomas regressed five weeks earlier than the controls. E7 vaccination achieved two goals: inhibition of papilloma development and early regression of the lesions.

Figure 3 shows the vaccination results. The numbers of papillomas of size greater than 2mm is higher for the control group than for the vaccinated group.

EXAMPLE 2 (Epitope Mapping of BPV-4 E7)

Vaccination of cattle with E7 is followed by vigorous humoral and cellular immune response to the vaccine. Both responses appear much earlier and have a greater amplitude in the vaccinated animals than in the control calves. Indeed, some of the control animals never developed detectable antibodies to E7 throughout the course of the experiment. The T cell response is particularly strong especially when compared to control animals and may explain the efficacy of the vaccine, although the possible contribution of anti-E7 antibodies cannot be discounted. Vaccine E7 is therefore successfully presented to both effector arms of the immune system while viral E7 is poorly presented. The immunodominant B- and T-cell epitopes encoded by E7 have been mapped by the use of synthetic overlapping peptides in ELISA and cell proliferation assays respectively. As shown in Figure 4 B1 maps to amino acids 1-30 at the N-terminus, B2 to amino acids 79-98 at the C-terminus and B3 to amino acids 51-69 in the middle portion of the protein. B1 and B2 are more often recognised than B3 (10/14, 11/14 and 6/14 animals respectively). T1 maps to amino acids 20-50 and T2 to amino acids 70-88; T1 appears to be recognised more often

than T2 (2/3 and 1/3 animals respectively). An interesting observation derives from these results. The regions of BPV-4 E7 where the epitopes have been mapped have amino acid and functional homology to HPV-16 E7 (Rb-binding domain I and II and Zn-binding domain) and the same areas in HPV-16 E7 have been shown to contain B- and T-cell epitopes (see Comerford et al., Krchnak et al., Tindle et al. (1990) and (1991)). The immunological homology between conserved areas of BPV-4 and HPV-16 E7 proteins gives weight to the suggestion that the former is a predictive indicator of the latter.

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SEQUENCE LISTING (FIGURE 2)

SEQ ID NO : 1

SEQUENCE TYPE : protein sequence

SEQUENCE LENGTH : 98 amino acids

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULAR TYPE : E7 protein

ORIGINAL SOURCE

ORGANISM : Bovine papilloma virus BPV-4

USE : therapy

DEPOSIT :

FEATURES aa 4-15 and 23-36 = presumed retinoblastoma protein binding domain

aa 47-50 and 80-83 = presumed zinc fingers domains

aa 54-73 = presumed hydrophobic stretch.

SEQUENCE LISTING (FIGURE 4)

SEQ ID NO : 2

SEQUENCE TYPE : protein sequence

SEQUENCE LENGTH : 98 amino acids

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULAR TYPE : E7 protein

ORIGINAL SOURCE

ORGANISM : Bovine papilloma virus BPV-4

USE : therapy

DEPOSIT :

FEATURES			
	aa 1-30	B1	epitope
	aa 21-49	T1	"
	aa 50-69	B3	"
	aa 70-88	T2	"
	aa 79-98	B2	"

CLAIMS

1. A pharmaceutical formulation for the therapy of papillomavirus disease, which comprises papillomavirus E7 protein or therapeutically effective fragment thereof in admixture with a pharmaceutically acceptable carrier.

2. A formulation according to claim 1 wherein the E7 protein is a bovine papillomavirus protein.

3. A formulation according to either preceding claim wherein the E7 protein is BPV-2 or BPV-4 protein.

4. A formulation according to any preceding claim comprising a therapeutically effective E7 fragment which has the sequence amino acids 1-30, 79-98, 51-69, 20-50 or 70-88 as set out in Figure 4;
or with additions, deletions or substitutions thereto which do not substantially effect the therapeutic effectiveness thereof.

5. A formulation according to any preceding claim wherein the E7 protein is present in the form of a fusion protein with a different co-protein.

6. A formulation according to claim 5 wherein the co-protein in the E7 fusion protein is beta-galactosidase.

7. A formulation according to claim 5 wherein the co-protein in the E7 fusion protein is glutathione S-transferase (GST).

8. A formulation according to any preceding claim wherein the E7 protein or fragment thereof is produced by recombinant DNA techniques.

9. A formulation according to any preceding claim in the form of an injectable formulation, wherein the carrier is a pharmaceutically acceptable injection vehicle.

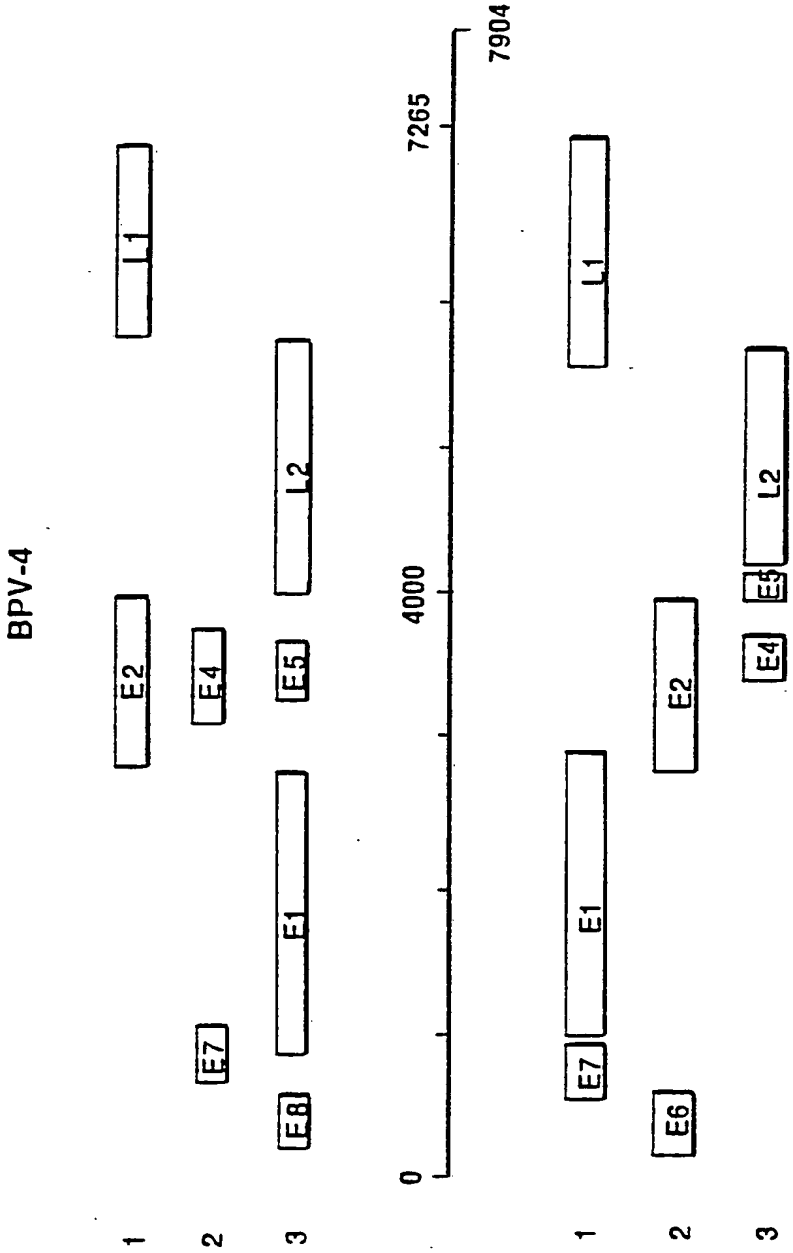
10. A formulation according to any preceding claim which further comprises an adjuvant selected from Freund's incomplete adjuvant, and aluminium gel.

11. A transformed bacterial cell producing recombinant E7 protein or therapeutically effective fragment thereof.

12. Use of papillomavirus E7 protein or effective fragment thereof in medicine for the therapy of papillomavirus disease.

13. Use of papillomavirus E7 protein or effective fragment thereof in the production of a medicament for use in the therapy of papillomavirus disease.

14. A method of treating a mammal for the therapy of papillomavirus disease, which comprises the administration of papillomavirus E7 protein or effective fragment thereof to the mammal in a therapeutically effective dosage.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E2
E4
L2
E5
E1
E7
E8
1
2
3
0
4000
7265
7904
L1
E1
E7
E2
E6
L2
E5
E4
HPV-16
1
2
3

FIG. 1

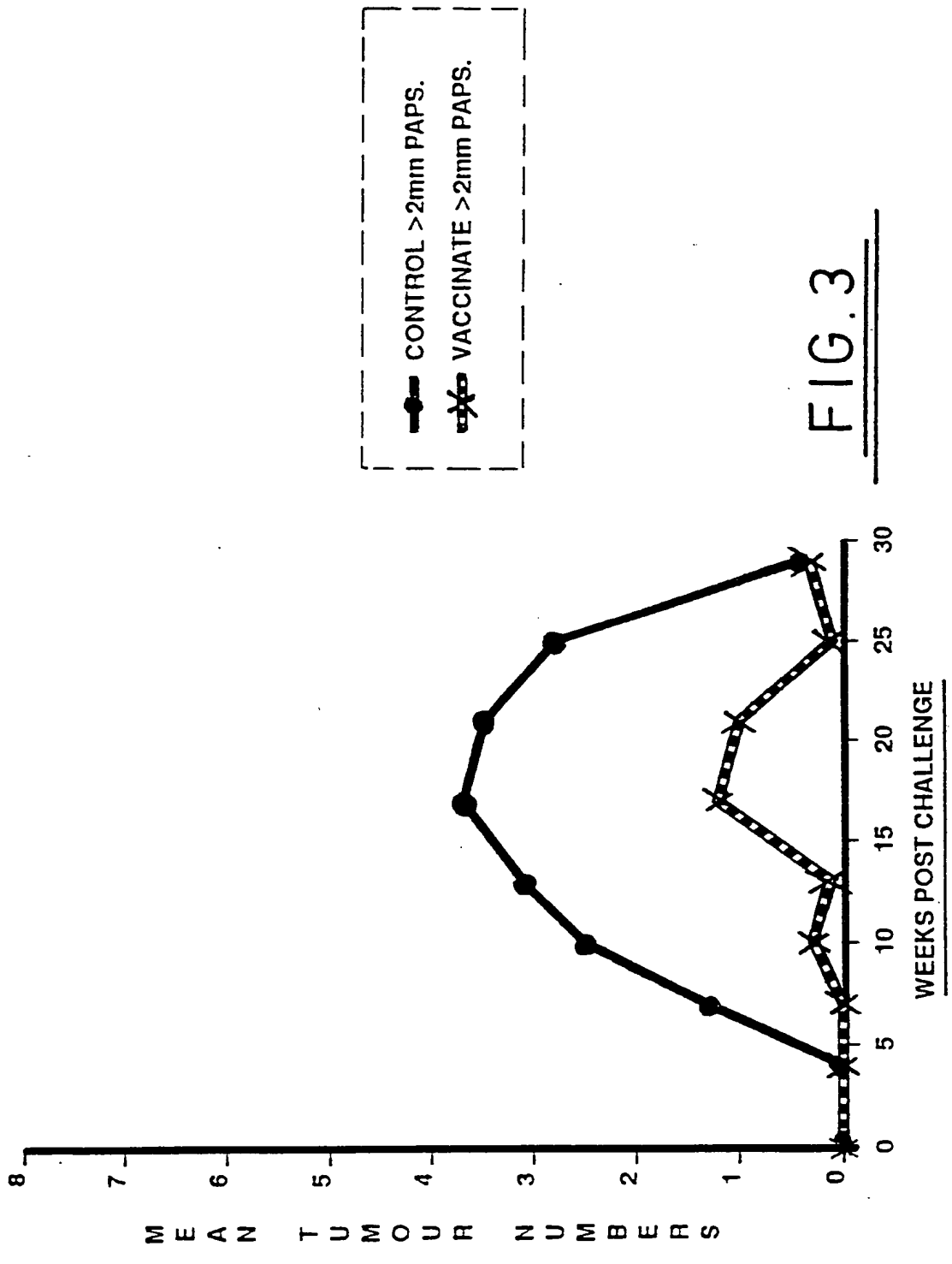
```
BPV4 E7  aa1  MKGQNVTLQD.IAIELEDTISPINLHCEEEI.E.TEEVDT.....PNP.FA
                |:|:  |||: : ::|:     :| | |:: : :|| |       | |
HPV16 E7 aa1  MHGDTPTLHEYM.LDLQ..PETTDLYCYEQLNDSSEEDEIDGPAGQAEPDRA

          Region 1          Region 2

                                  ↱
BPV4 E7  aa43 ...ITATCYACEQVLRLAVVTST.EGIHQLQQLLFDNLFLLCAACSKQVFCNRRPERNGP
                 |  |: |:  |||  | ||   |: |::||:  | ::| :||
HPV16 E7 aa51 HYNIVTFCCKCDSTLRL.CVQSTHVDIRTLEDLLMGTLGIVCPICSQKP

          Zn++      hydrophobic      Zn++
```

FIG. 2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VACCINATE >2mm PAPS.
MEAN TUMOUR NUMBERS
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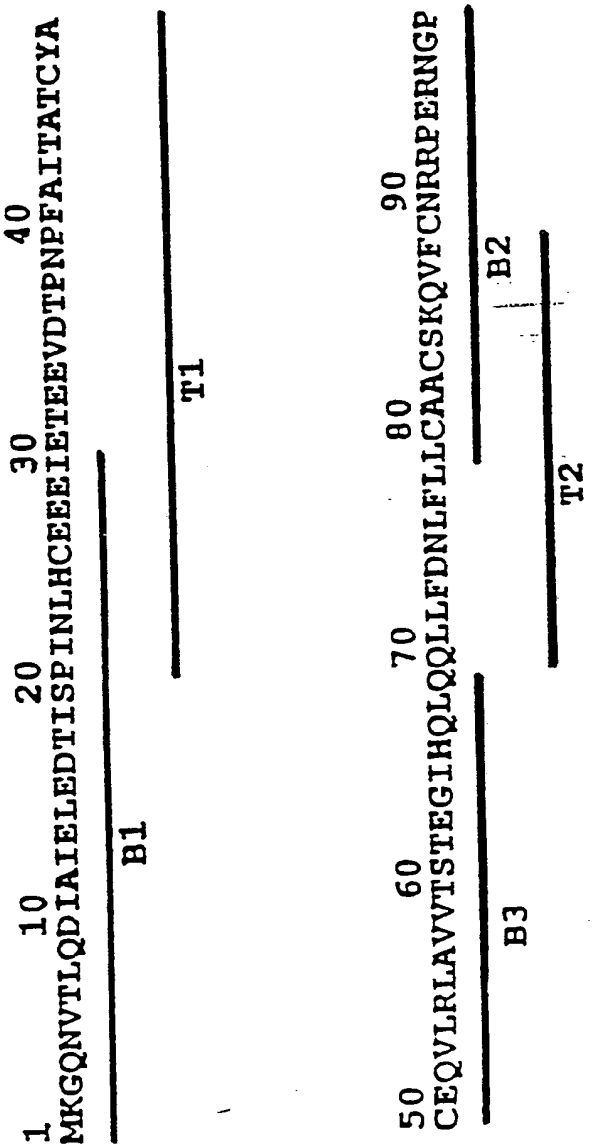
8
0
5
10
15
20
25
30
WEEKS POST CHALLENGE

FIG. 3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B1
T1
50 60 70 80 90
CEQVLRLAVVTSTEGIHQLQQLLFDNLFLLCAACSKQVFCNRRPERNGP
B3
T2
B2

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00679

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K39/12; C12N1/21; //C12N15/37

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K ; C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X,P	WO,A,9 300 436 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED) 7 January 1993 cited in the application see page 14 - page 15; example 2 ---	1-11,13
X,P	EP,A,0 531 080 (MERCK & CO. INC.) 10 March 1993 see page 3, line 3 - page 5, line 27 ---	1,8,11, 13
X	WO,A,9 205 248 (BRISTOL-MYERS SQUIBB COMPANY) 2 April 1992 cited in the application see page 23, line 6 - page 31, line 6 ---	1,8,11, 13
X	EP,A,0 451 550 (BEHRINGWERKE) 16 October 1991 see page 1, line 1 - page 2, line 6 ---	1,8,13

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° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02 AUGUST 1993	20 -08- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT FFICE	REMPP G.L.E.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EP,A,0 456 197 (BEHRINGWERKE) 13 November 1991 see page 1, line 29 - line 54	1,8,13
A	THE JOURNAL OF GENERAL VIROLOGY vol. 68, no. 8, August 1987, pages 2117 - 2128 KAMALESH R. PATEL ET AL. 'THE NUCLEOTIDE SEQUENCE AND GENOME ORGANIZATION OF BOVINE PAPILLOMAVIRUS TYPE 4.'	

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/GB93/00679

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Altnough claims 12, 14 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300679
SA 72122

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 02/08/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9300436	07-01-93	AU-A-	1985992	25-01-93
EP-A-0531080	10-03-93	None		
WO-A-9205248	02-04-92	AU-A-	8762991	15-04-92
		CN-A-	1067382	30-12-92
EP-A-0451550	16-10-91	AU-A-	7351591	26-09-91
		JP-A-	4217998	07-08-92
EP-A-0456197	13-11-91	DE-A-	4015044	14-11-91
		AU-A-	7621291	14-11-91
		JP-A-	4227000	17-08-92

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82